Immunohistochemical Interplay in Associated Large Vessel Vasculitis and consequent Ascending Aortic Aneurysm and Review of Literature

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Large vessel vasculitis (LVV), such as giant cell arteritis (GCA) and Takayasu's arteritis (TA), are autoimmune diseases characterized by granulomatous inflammation of large vessels, including the aorta and its branch vessels. Disease etiology is still unknown. Comparative immunohistochemical (IMH) studies between GCA and TA cases might be important. The purpose of the study was to assess and to compare composition and distribution of the inflammatory infiltrate of the ascending aortic aneurysmal wall. Ascending aortic aneurysm (AAA) tissue blocks belonging to one male patient of 59 years and one female patient aged 36 years, diagnosed with GCA and TA, respectively, were used for histological and IMH analysis. Immunohistochemistry staining was performed using antibodies directed against markers of lymphocytes and macrophages. Histological analysis showed in both cases lymphocytes, macrophages, epithelioid histiocytes and multinucleated giant cells that infiltrate all layers of the arterial wall, intimal hyperplasia and adventitial fibrotic changes of different degrees. Immunohistochemical examination showed that the media and adventitia from GCA aortitis case had increased number of T cells and macrophages compared with TA aortitis case, and a less number of B cells in both. Our study made a morphologic and morphometric analysis of lymphocyte infiltration within aneurysm aortic tissue and demonstrated that immune inflammation is a typical feature of GCA and TA diseases. We also noted the utility of immunohistochemical staining of the aortic biopsies for evaluation of indeterminate or healing GCA and TA cases.

Keywords:immunohistochemical, granulomatous inflammation, aortic aneurysm, large vessel vasculitis

Large vessel vasculitis (LVV) is characterized by immune-mediated injury of predominantly large vessels. Histopathologic lesions contain mononuclear cell infiltration of the vessel wall that often includes granuloma formation. Within this group of diseases, Takayasu's arteritis (TA) affects younger individuals (under 50 years), whereas giant cell arteritis (GCA) is common in patients over 50 years. Both LVV predominantly affect women [1]. It has been suggested that GCA and TA may be the same disease with the same etiology, but with phenotypic variations due to immune and aortic aging substrate [2, 3]. The diagnosis is difficult because aortitis rarely has specific symptoms and diagnosis is made often by histopathology. The occurrence of concomitant aortitis and AAA are common forms of presentations in both diseases [4, 5]. Giant cell arteritis and TA are two granulomatous vasculitis which develop on a specific genetic background and share some similarities regarding the immunological pathways involved in their pathogenesis [6]. Generally, it is supposed that aortitis is the result of stimulation from an antigen of unknown nature, possibly infectious type. It seems that GCA could be initiated by exogenous antigens like viruses and bacteria, including parainfluenza virus, varicella zoster virus, Chlamydia pneumoniae, and Mycoplasma pneumoniae [7]. Similarly, TA patients had an increased immune response

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to Mycobacterium tuberculosis [8]. In addition it might be initiated in the adventitia, with inflammatory cells entering through the vasa vasorum and subsequently infiltrating into all layers of the aortic wall [9]. We aimed to analyze and compare immunohistochemical (IMH) of two cases, in order to better understanding these pathologies.

Experimental part

Material and methods

The study was approved by our institutional Committee of Ethics. Written informed consent was obtained from the patients.

The two cases of TA and GCA patients were one female under <50 years old and one male over > 50 years old, respectively. The study material, the residual histological blocks, were used for histological and IMH comparative examination. These cases were selected from 10 cases of AAA associated with aortitis, both having at least 3 positive criteria of diagnosis according with ACR aortitis classification.

We analyzed the aortic morphology on hematoxylin-eosin (HE) and elastic tissue fibers - Verhoeff's Van Gieson (EVG) sections by using an Olympus microscope (model CX41; Olympus, Tokyo, Japan). Morphometric analysis of macrophages (CD68) and T-lymphocytes (CD3) and B-lymphocytes (CD20) was accomplished with imaging analysis software (QuickPHOTO MICRO 3.0).

Histologically, we analyzed 10 histological fields at a magnification of x200, for each case. We measured the wall thickness of the aortic wall. The measurements were expressed in mean values.

We analyzed immunohistochemically 10 histological sections at a magnification of x200 for each case. The degree of total wall inflammation was evaluated by relating the average inflammatory cell number to the entire studied histological section area. Data were expressed as mean values.

For the evaluation of each inflammatory cell component of the infiltrate we calculated the proportion of each cell related to total inflammatory cell number in the same histological section area. The results were expressed as percentage.

The degree of macrophages, B and T cells was morphologically classified by inflammatory cell distribution in the aortic tissue, as follows: grade 0 (<10%), grade 1 (between 10-50%), grade 2 (between 50-75%), and grade 3 (over > 75%). The end inflammatory score was the sum of the score for each type of inflammatory cell. Finally, we compared the score results.

Statistical analysis

The IMH comparison of composition and distribution of the inflammatory infiltrate of the AAA wall between GCA and TA case was assessed. Data are expressed as mean values and percentages, calculated using Excel software (Microsoft Corporation, Redmond, WA, USA).

Results and discussions

The average values of aortic thickness (intima, media, and adventitia) in the GCA and TA are presented in Table 1. The aortic wall thickness was about double in TA (4034 μm) comparative with GCA (2122 μm). The medial layer of GCA was thicker than that of TA, while the adventitia was thinner, respectively.

Histologically, in GCA we found a fibrous extracellular matrix intima containing foci of mononuclear inflammatory cells located mainly at I-M junction, medial fibrous areas and nodular and band-like inflammatory foci in association with neovascularization, and adventitial nodular and band-like inflammatory foci and intense neovascularization (Fig. 1a).

In TA, we noted a thickened intima by collagenous bands disposed parallel to the endothelium, medial areas of elastic lamina destruction and nodular and band-like inflammatory foci in association with neovascularization, and thickened adventitia by connective extracellular matrix and neovascularization (Fig. 1b).

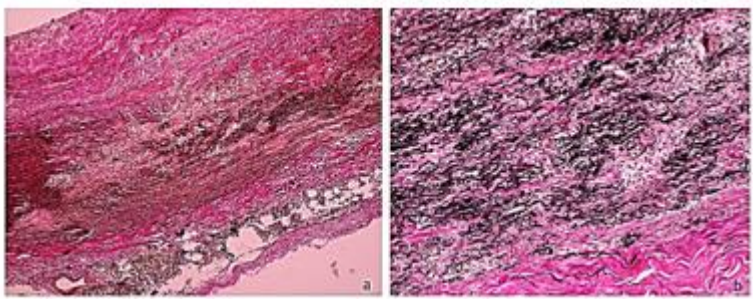

Fig. 1. a. Giant cell arteritis aortic wall x100 EVG (elastic tissue fibers – Verhoeff's Van Gieson);b. Takayasu's arteritis aortic wall x200 EVG.

Distribution of inflammatory cells within aortic layers, in giant cell arteritis (GCA) and Takayasu's arteritis (TA) are showed in Table 1.

Table 1
IMMUNOHISTOCHEMICAL ASSESSMENT OF INFLAMMATION IN GIANT CELL ARTERITIS (GCA) AND TAKAYASU'S ARTERITIS (TA)

	GCA				TA			
Histo-pathologic staining	Intima	Media	Adventitia	Total	Intima	Media	Adventitia	Total
Average aortic wall thickness (µm)	546	1045.5	530.5	2122	662	1405	1967	4034
Layer thickness proportion (%)	0.26	0.49	0.24	100%	0.17	0.35	0.48	100%
Average of total inflammatory cell number	221	276	620	1117	19	594	345	958
GCA/TA inflammation ratio	11.63	0.46	1.79	1.16				

In the aortic wall, inflammatory cells are of 1.16 times more in GCA (1117) than in TA (958), and with different values in the layers of the aortic wall. Inflammatory infiltrate is of 11.6 higher in the intima of the aorta in GCA (221) than in TA (19) and only of 1.79 times higher in aortic adventitia in GCA (620) than TA (345).

Inflammatory cell ratio was approximately double (2.15) in media of the aortic wall in TA (594) than GCA (276) or less than half times less (0.46) in GCA than in TA.

So, our results showed that both aortitis are panarteritis, with similar histology and different distribution and degrees of inflammation within aortic all. In our opinion, the inflammation in both aortitis is a chronic phase with still persistent inflammation in TA.

The proportion of inflammatory cells within aortic wall layers is showed in Table 2.

Table 2
PROPORTION OF INFLAMMATORY CELLS WITHIN AORTIC WALL LAYERS.
GCA: GIANT CELL ARTERITIS; IMH: IMMUNOHISTOCHEMICAL; TA: TAKAYASU'S ARTERITIS

GCA and TA - IMH staining's (number, %)	**CD3**	**CD20**	**CD68**	**Total inflammatory cell number**
GCA				
Intima cells (%)	21	8.5	70.5	221
Media cells (%)	39.2	8.6	52.2	276
Adv. cells (%)	45.7	33.5	20.8	620
Total aortic wall cell (%)	**39.3**	**22.4**	**38.3**	**1117**
TA				
Intima cells (%)	5.26	-	-	19
Media cells (%)	60.2	28.2	11.6	594
Adv. cells (%)	61.1	14.9	24	345
Total aortic wall cells (%)	**61.5**	**22.8**	**15.7**	**958**

In GCA, the inflammatory infiltrate (1117 cells) contained 39.3% T - lymphocytes (CD3), 22.4% B - lymphocytes (CD20) and 38.3% macrophages (CD68), while in TA, the inflammatory infiltrate (958 cells) contained 61.5% T - lymphocytes (CD3), 22.8% B - lymphocytes (CD20) and 15.7% macrophages (CD68).

Comparative layer inflammatory distribution in GCA vs TA was the following:

-In GCA, intima contains 221 mononuclear inflammatory cells, of which 21% are T - lymphocytes, 8.5% are B - lymphocytes and 70.5% are macrophages while in TA, all 19 inflammatory cells were T - lymphocytes located only in the intima.

-The medial layer contains in GCA 276 mononuclear inflammatory cells, of which 39.2% are T - lymphocytes, 8.6% are B - lymphocytes and 52.2% are macrophages, while in TA of 594 mononuclear inflammatory cells, 60.2% are T - lymphocytes, 28.2% are B - lymphocytes and 11.6% are macrophages.

- In GCA, adventitia contains 620 mononuclear inflammatory cells, of which 45.7% are T - lymphocytes, 33.5% are B - lymphocytes and 20.8% are macrophages, while in TA of 345 mononuclear inflammatory cells, 61.1% are T - lymphocytes, 14.9% are B - lymphocytes and 24% are macrophages.

Entire aortic wall inflammatory score in GCA and TA is showed in Table 3. Total wall inflammatory infiltrate score was bigger in TA (4) than in GCA (3).

Individual inflammatory cell score in GCA and TA is showed in Table 4.

Table 3
TOTAL WALL INFLAMMATORY SCORE IN GCA AND TA. GCA: GIANT CELL ARTERITIS; LYS: LYMPHOCYTES; TA: TAKAYASU'S ARTERITIS

	T - Lys (%)	grade	B- Lys (%)	grade	Macrophages (%)	grade	Sum Score
GCA inflammatory cell (N=1117)	39.3	1	22.4	1	38.3	1	3
TA inflammatory cell (N=958)	61.5	2	22.8	1	15.7	1	4

Table 4
INFLAMMATORY CELL SCORE IN GIANT CELL ARTERITIS (GCA) AND TAKAYASU'S ARTERITIS (TA) WITHIN AORTIC WALL LAYERS

GCA and TA inflammatory cell % & score	CD3 %-score		CD20 %-score		CD68 %-score		Total score
GCA							
Intima	21	1	8.5	0	70.5	2	3
Media	39.2	1	8.6	0	52.2	2	3
Adventitia	45.7	1	33.5	1	20.8	1	3
Total GCA wall inflam. score		3		1		5	9
TA							
Intima	5.26	0	-	-	-	-	0
Media	60.2	2	28.2	1	11.6	1	4
Adventitia	61.1	2	14.9	1	24	1	4
Total TA wall inflam. score		4		2		2	8

The inflammatory infiltrate had a similar distribution in the aortic all, with different proportion of the inflammatory cells (Fig. 2.).

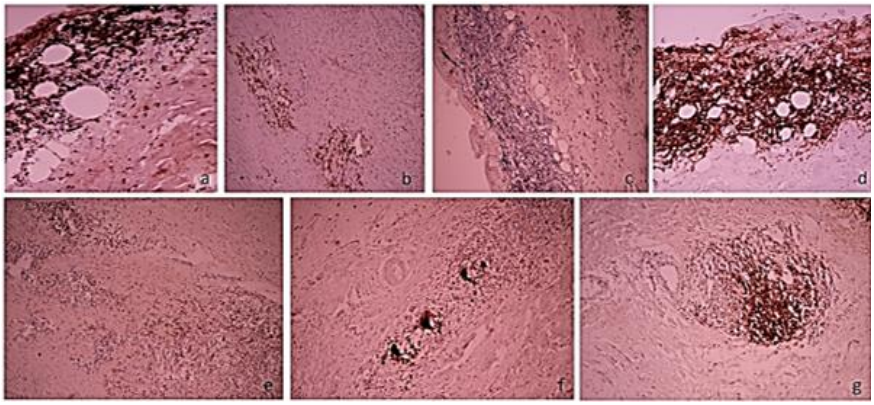

Fig. 2. Giant cell arteritis:a-adventitial CD3; b-medial CD3; c-medio-adventitial CD68; d-adventitial CD20. Takayasu's arteritis: e-intimo-medial CD3;f-medio-adventitial CD68; g-adventitial CD20

Comparative inflammatory score in GCA and TA was assessed according with two criteria. Depending on the aortic wall involved layers, the total inflammatory score in GCA was 9, the same in intima (3), media (3) and adventitia (3), while the total inflammatory score in TA was 8, having 0 value in intima and 4, in both media and adventitia. Depending on the type of inflammatory cells in the entire wall thickness, the inflammatory score in GCA was 3 for T – lymphocytes (CD3), 1 for B - lymphocytes (CD20) and 5 for macrophages (CD68), while the inflammatory score in TA was 4 for T – lymphocytes, 2 for B - lymphocytes and 2 for macrophages.

In GCA, the proportion of the inflammatory cells of 3:1:5 probably denotes a chronic healing process with a mild recent activation by an exogenous agent, maybe viral one. In TA, the proportion of the inflammatory cells of 4:2:2 probably denotes a persistent chronic inflammation which is still active (due to persistence of antigen stimulation related by tuberculosis history), demonstrated by extensive destructive areas. Both aortitis have about the same inflammatory score, but they are in various evolutionary stages related to different intensity of inflammatory activity.

Takayasu arteritis and GCA are the two main variants of LVV. Takayasu's arteritis is a LVV affecting elastic arteries such as the aorta and its branches, while GCA refers to involvement of the aorta and its proximal or extracranial aortic branches. In a patient with GCA, Prieto-Gonzalez showed the aortic involvement using computed tomography angiography (angio-CT) and aortic biopsy [10].

These two conditions have been considered separate entities because of the observed differences in the age at onset, showing clinical features, geographic distribution and location of arterial involvement (8). Recently, Polachek A et al have proposed that TA and GCA may exist within a clinical spectrum of a single disease [11].

Both our cases had AAA and aortic insufficiency at hospital presentation, having symptoms related to this vascular disease. Both LVV cases had positive inflammatory markers and angiographic signs of ascending aortic involvement, completed with specific signs of aortic branch stenosis in TA case. The artery biopsy was the gold standard for diagnosis of these aortitis [12].

According with Eberhardt RT (13) et al, we also found at GCA histology exam a chronic evolutionary stage of the GCA characterized by moderate intimal thickening by fibromyxoid changes, medial granulomas with rare multinucleated giant cells and medial fibrosis, and adventitial fibrosis. The inflammation showed to be marked in the adventitia, too [13].

As Vaideeswar P notes, we also reported at histological TA exam a chronic evolutionary stage, consisting in thicker, collagenous intima, medial focal granulomas related to elastic fibers fragmentations and medial fibrosis, and focal adventiceal perivasa vasorum inflammatory infiltrate. In addition, we also noted neovascularization within the entire wall [14].

Due to similar GCA and TA histopathological findings, the biopsy results only may not differentiate between these two vasculitis. So, in discrimination of the two aortitis, the age in both and CT angiography in TA are essential tools.

Similar with Chakravarti R appreciation, we consider that the more severe medial inflammation leads to loss of smooth muscle cells and elastic fibers, medial weakening and aneurysm formation. In addition, we revealed a significant adventitial reactive fibrosis in the intima, media and adventitia and neovascularization at the medio-adventitial junction in TA [15].

In both TA and GCA we found parietal inflammatory lesions produced in the adventitia through the vasa vasorum, allowing the lymphocytes to gain access to the arterial wall, as Noris M. also observed [16].

Cell-mediated autoimmunity has been clearly involved in the physiopathology of vascular cell injury in TA and GCA, due to the highest values of T - lymphocytes and macrophages in these two diseases. We did not found GCA associated infections (a virus infection cannot be excluded) while we noted a history of tuberculosis infection in TA patient.

Furthermore, an increased immune response to Mycobacterium tuberculosis has been reported by Moraes MF in TA cases, too (8). We consider, that using lymphocytic and histiocytic markers (CD68) in suspected GCA and TA, we can detect residual arteritis in patients with resolving disease.

If humoral immunity takes part in the physiopathology of GCA and TA is not clear. We found more B Lys in GCA than TA, and we consider that both aortitis have a persistent active inflammation of different degrees.

LVV adventitial analysis showed that inflammation was most prominent in GCA, with infiltration of B and T cells. In TA, we found adventitial inflammatory foci, with B and T cells surrounding vasa vasorum. The distribution of immunostainings seen at the outer media border indicates that the majority of the inflammatory cells enters the arterial wall from adventitial microvessels, migrate through the media and the inflammatory activity focuses finally on the media and intima.

Conclusions

This study showed that beyond the role of T cells in GCA and TA, B cells are also involved in humoral responses in patients with activated disease. The initial site of inflammation seems to be around the adventitial vasa vasora which is an early stage in the development of classic transmural inflammatory infiltration. The medial inflammatory areas related to prominent elastic fibers fragmentation was the cause of medial weakening and aneurysm formation.

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